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Carbon Dioxide and Oxygen-Nitrogen Ratios as Factors Affecting Salmon Survival in Air-Supersaturated Water

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ABSTRACT

Juvenile sockeye salmon (*Oncorhynchus nerka*) were exposed to lethal levels of air-supersaturated water (120 percent, 125 percent, 130 percent total gas saturation) containing different oxygen-nitrogen ratios and different carbon dioxide concentrations. Fish mortality was not significantly different at different carbon dioxide levels (1.7 to 22.0 mg/liter CO_2) when tested at the same total gas saturation concentrations. Total gas saturation levels are much more important than the O_2/N_2 ratios, as fish mortality will not occur unless total gas saturation exceeds 100 percent, regardless of the O_2/N_2 ratios. There was a significant decrease in mortality when the ratio of oxygen to nitrogen was increased while holding the total percent saturation constant. Much more extensive and severe signs of gas bubble disease developed at high O_2/N_2 ratios than at low O_2/N_2 ratios, indicating that oxygen plays a significant part in forming external emphysema and lesions.

Dissolved gas composition in surface waters can vary for several reasons including photosynthetic activity, biochemical oxygen demand, aeration activity at dams, and upwellings. The concentrations of inert gases tend to change rather slowly in water while carbon dioxide and oxygen, which are acted upon biologically, will fluctuate significantly. Surface and underground water can have widely different gas saturation levels depending upon water source, temperature, turbulence, pressure, presence of oxygen-consuming materials, man's activities such as dams, and other factors. Recent studies (Nebeker and Brett 1976) have shown that juvenile sockeye salmon die rapidly at total gas levels of 120 percent–130 percent. Other studies with salmon and other fish species are summarized in the literature reviews of Rucker (1972), Weitkamp and Katz (1973, 1975), and Bouck (1973). However, the literature contains little information on how carbon dioxide or oxygen-nitrogen ratios affect the lethality of gas-supersaturated water to freshwater or marine fish or invertebrates.

Carbon dioxide can cause adverse physiological effects in fish at levels as low as 30 ppm, and most effects occur at levels less than 10 percent of saturation, far below levels which could be expected to cause formation of bubbles (Harvey 1975). Mrsic (1933) attributed gas bubble formation to CO_2, but no other reports have been found that confirm his studies.

Rucker (1972) and Harvey (1975) have reviewed the literature on effects of oxygen causing gas bubble disease. Rucker states that oxygen can cause gas bubble disease from the effect of the partial pressures of oxygen and nitrogen. If excess oxygen is present in water with a deficiency of nitrogen the total gas pressure may be too low to cause a problem, but as Egusa (1969) indicates, excess oxygen can cause a problem if nitrogen is maintained normal. The sum of the partial pressures must be greater than the ambient pressure to cause gas bubble disease. Harvey (1975) in his review summarizes by stating that there is no known naturally occurring case of gas disease due to oxygen alone; in reported cases attributed to oxygen only oxygen was measured. Oxygen supersaturation was probably superimposed on air-saturated or supersaturated waters, in many cases caused by increased water temperatures. Whether nitrogen was supersaturated or not, bubble formation and growth would involve both oxygen and nitrogen.

Many studies (Weitkamp and Katz 1973, 1975) have shown that water supersaturated with nitrogen causes severe damage and

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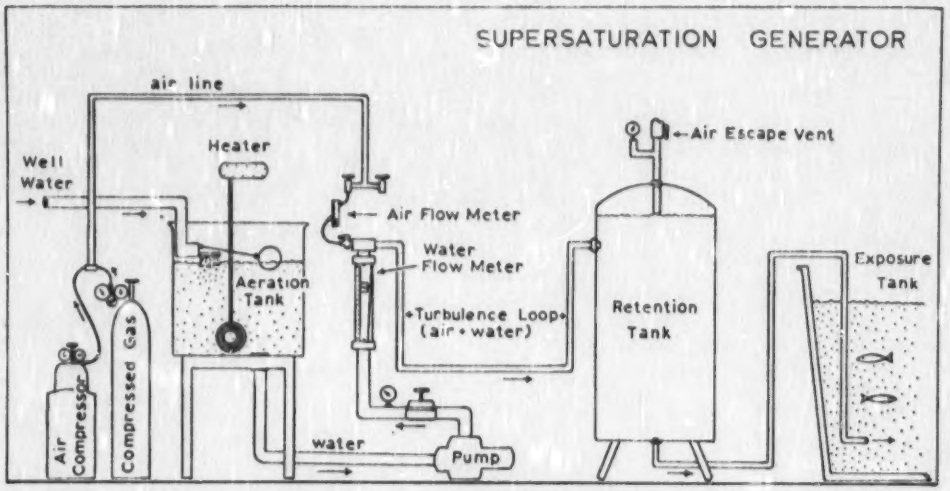

FIGURE 1.—*Diagram of system for adding oxygen or carbon dioxide to air-supersaturated water generator facility.*

death to a variety of fish species. However, only Rucker (1976) has conducted detailed studies to show the effect of varying oxygen-nitrogen ratios on fish while maintaining a constant total gas pressure. Rucker held the total gas saturation constant at 119 percent and varied the oxygen-nitrogen ratio, using juvenile coho salmon as the test species. The oxygen-nitrogen ratios varied from 50 percent/138 percent to 229 percent/90 percent. His data indicated that total gas supersaturation had to occur before fish damage occurred and that oxygen plays a more significant role above 110 percent than below 110 percent total gas saturation. A drastic decrease in lethal effect of individual ratios of O_2 to N_2 occurred between 159 percent O_2/109 percent N_2 and 173 percent O_2/105 percent N_2 at the same total gas pressure (119 percent).

The purpose of this study was to determine if (1) increased concentrations of carbon dioxide within normal extremes found in the field, and (2) different oxygen-nitrogen ratios, would cause differences in survival and signs of gas bubble disease in young sockeye salmon (*Oncorhynchus nerka*) at constant levels of air-supersaturated water.

METHODS

Physical Conditions

The test facilities used during these studies were the same as those described by Nebeker et al. (1976), where four 6,000-liter fiberglass tanks were used as test containers and individual supersaturation generators produced test water for each tank. One generator was modified to permit addition of CO_2 or O_2 gas to the compressed air which is added to the test water. Compressed CO_2 or O_2 at 2.8 kg/cm^2 was carefully metered into the compressed air line (2.7 kg/cm^2). This gas mixture was then metered and injected into the water supply which was pumped at a pressure of 1.7 kg/cm^2. The mixture of compressed air, CO_2 (or O_2) and water was then passed through a detention tank where excess gas was vented at the top and the supersaturated water was drained from the bottom and delivered to the test tanks (Fig. 1).

Two test tanks were used for control tanks with supersaturated water but without the addition of extra CO_2 or O_2. One tank was modified for the addition of extra CO_2 or O_2 into supersaturated water, and the fourth

tank was used for an unsupersaturated water control tank. Fish were tested either in the open tank or in four cages set inside the tank. Four groups of fish could be tested simultaneously in each of the four tanks.

The test water was obtained from two wells located about 30 m from the Willamette River. The water was chilled to just below test temperature, aerated to saturation to stabilize the dissolved gas concentrations, and then maintained at final test temperature with electric immersion heaters. Well water had low dissolved oxygen concentrations (3.0–5.0 mg/liter) making it very easy to get low oxygen to nitrogen rations without adding N_2 gas in addition to O_2 gas. This was accomplished by decreasing aeration of the well water. Photoperiod was natural for the time of year because the tanks were shaded and out-of-doors.

Chemical and Gas Analyses

The well water used for tests was relatively soft with the following average chemical characteristics: hardness, 34 mg/liter; alkalinity (as $CaCO_3$), 31 mg/liter; pH, 6.7–7.3 (depending upon aeration time). Complete chemical characteristics of the test water can be found in the publication by Samuelson (1976). All chemical analyses were conducted according to the American Public Health Association (1971). Dissolved oxygen was measured by the azide modification of the Winkler method. Hydrogen ion (pH) concentration was measured with glass electrode pH meters. Hardness was determined using the EDTA titrametric method, and alkalinity was determined potentiometrically with 0.02 N H_2SO_4 and colorimetrically. Carbon dioxide (as free CO_2) was calculated nomographically, and determined potentiometrically and colorimetrically. The means of these results were used as final CO_2 values.

Percent total air saturation was determined with a Weiss saturometer (Nebeker et al. 1976), calibrated with the Van Slyke gas analyser and the Winkler method for oxygen. The formula

$$\frac{BP + \Delta P - VP}{BP} \times 100 = \% \text{ saturation},$$

where BP = barometric pressure, ΔP = saturometer reading, and VP = water vapor pressure, was used to calculate total percent saturation.

Percent oxygen saturation was determined by converting the oxygen in mg/liter from the Winker analysis to ml/liter, and then dividing by the O_2 saturation at the given temperature obtained from tables by Weiss (1970):

$$\frac{O_2 \text{ mg/liter} \times 0.7}{\text{Sat. } O_2 \text{ @ Temp.}} \times 100 = \%\ O_2 \text{ saturation.}$$

Percent carbon dioxide saturation was determined using 2,260 mg/liter CO_2 at 15 C as saturation and dividing that into the actual mg/liter CO_2 determined in test water.

Percent nitrogen (including argon) saturation was determined following the method of Lambert and Rulifson (1972):

$$\text{Step 1. } PO_2 = \frac{O_2 \text{ mg/liter } (0.7)}{BO_2} \times 0.76$$

$$= O_2 \text{ in mm Hg.}$$

$$\text{Step 2. } PN_2 = BP + \Delta P - (PO_2 + PH_2O)$$

$$= N_2 \text{ in mm Hg.}$$

$$\text{Step 3. } \%\ N_2 = \frac{PN_2}{0.79\,BP} \times 100$$

$$= \%\ \text{nitrogen (+ argon)},$$

where PO_2 = oxygen pressure in mm Hg; BO_2 = Bunson coefficient for oxygen (Weiss 1970); PN_2 = nitrogen (+ argon) pressure in mm Hg; BP = barometric pressure in mm Hg; ΔP = gauge reading on saturometer in mm Hg; PH_2O = water vapor pressure in mm Hg (Weiss 1970); and 0.79 = % N_2 + Ar in dry air.

Fish Test Methods

Young sockeye salmon (*O. nerka*) nine to twelve months old, were tested with normal and high levels of CO_2 and various O_2/N_2 ratios at 120 percent, 125 percent, and 130 percent total gas saturation. They were chosen as the test fish because sockeye are sensitive to supersaturation (Nebeker and Brett 1976) and they are a different species

than tested by Rucker (1976). The fish were hatched and reared from eggs taken from adults collected at the north ladder of Bonneville Dam on the Columbia River and artificially fertilized at Western Fish Toxicology Station, Corvallis, Oregon. The fish hatched in December, 1972, and were tested September–December, 1973. Fish were fed Oregon Moist Pellets during testing. Mean fork-length and weight of fish during testing were 8.6 cm and 7.7 g, respectively.

Five tests were conducted, each using 160 fish. Two tests were conducted with a high O_2/N_2 ratio and three tests were completed with a high CO_2 level. Ten fish were placed in each chamber of the four-chambered cage in each tank, totalling 40 fish at each gas level (each tank) and O_2 or CO_2 level. Test water was 60 cm deep and held at 16 ± 1 C for the CO_2 tests and 15 ± 1 C for O_2/N_2 ratio tests. All fish were randomly assigned to test chambers and acclimated to test facilities for two weeks prior to testing in test 1, three days in tests 2 and 4, and placed directly into supersaturated water in tests 3 and 5.

Time to 50 percent death at each concentration was determined and the t test of significance ($P = 0.05$) was used to compare survival of young sockeye at different CO_2 levels and O_2/N_2 ratios.

RESULTS AND DISCUSSION

Effects of CO_2

Fish tested at 10.0, 2.8, and 2.1 mg/liter free CO_2 at 125 percent total gas saturation showed no significant difference ($P = 0.05$) in survival times, although the fish at 10 mg/liter did not die as rapidly. Time to 50 percent death did not differ significantly for fish tested at 20.0, 2.6, and 1.7 mg/liter CO_2 with 120 percent total gas saturation and essentially identical nitrogen saturation levels. Fish held at 22 mg/liter CO_2 with 120 percent total gas saturation also showed no significant difference in survival from those held at 3.3 and 2.1 mg/liter CO_2. No deaths occurred in the control fish during testing (Table 1).

TABLE 1.—*Effect of CO_2 on survival of juvenile sockeye salmon in supersaturated water at 125 percent and 120 percent and 16 ±1 C.*

Total percent saturation Mean ± SD	Mean CO_2 percent saturation	Mean O_2 percent saturation / Mean N_2 percent saturation (O_2/N_2 ratio)	Mean CO_2 (mg/liter)	Mean time to 50% death[a] (hours)
127.1 ± 0.6	0.4	126.5 / 127.9	10.0	12.1
127.2 ± 1.5	0.1	120.3 / 129.7	2.8	10.0
127.8 ± 1.4	0.1	126.4 / 128.9	2.1	9.2
120.0 ± 0.6	0.9	109.6 / 123.3	20.0	57.8
120.5 ± 0.5	0.1	113.6 / 123.1	2.6	46.5
120.0 ± 1.4	0.1	114.6 / 122.0	1.7	52.8
119.8 ± 0.9	1.0	116.5 / 121.2	22.0	73.2
119.9 ± 0.8	0.1	114.6 / 121.8	3.3	51.3
119.5 ± 1.0	0.1	119.7 / 119.9	2.1	56.3

[a] No significant differences ($P = 0.05$) between survival at high and low CO_2 levels.

Effects of O_2/N_2 Ratios

Total gas saturation levels are much more important than the O_2/N_2 ratios, as fish mortality due to gas bubble disease will not occur unless total gas saturation exceeds 100 percent, regardless of the O_2/N_2 ratios. There was a significant ($P = 0.05$) decrease in mortality when the ratio of oxygen to nitrogen was increased while holding the total percent saturation constant. Fifty percent died after 71 hours at 120 percent when the O_2/N_2 ratio was 117.2/121.3 percent. Only 7 percent of the fish died after 167 hours when the O_2/N_2 ratio was changed to 170.5/107.0 percent. No deaths occurred in the control fish.

Fish tested at 130 percent total gas saturation also exhibited significant differences ($P = 0.05$) in time to 50 percent death at different O_2/N_2 ratios. Mean time to 50

TABLE 2.—*Effect of O_2/N_2 ratios on survival of juvenile sockeye salmon in supersaturated water at 130 percent and 120 percent at 15 C.*

Total percent saturation Mean ± SD	Mean O_2 percent saturation / Mean N_2 percent saturation (O_2/N_2 ratio)	Mean O_2 (mg/liter)	Mean time to 50% death (hours)
129.4 ± 1.5	186.2 / 114.6	18.7	37.0[a]
129.9 ± 1.0	129.5 / 130.1	13.1	22.3
129.7 ± 1.4	127.5 / 130.5	12.8	23.3
120.1 ± 0.3	170.5 / 107.0	17.2	[a,b]
120.3 ± 0.8	117.2 / 121.3	11.8	71.6
120.1 ± 0.4	114.5 / 121.8	11.5	65.8

[a] There was a significant difference ($P = 0.05$) between survival at high and low O_2/N_2 ratios.

[b] Seven percent dead after 167 hours.

percent death was 37.0 hours at 186.2/114.6 percent and 22.3 hours at 129.9/130.1 percent (Table 2).

External Signs of Gas Bubble Disease

External signs of gas bubble disease did not appear until several hours after initial exposure to gas-supersaturated water and their severity was closely correlated with percent saturation, O_2/N_2 ratios, and exposure time. At 125 percent total gas saturation most external signs of GBD consisted of bubbles (emphysema) in the tail. Some bubbles occurred on the paired, dorsal, and anal fins, but most of the fish died before extensive emphysema developed. The fish held at 120 percent saturation exhibited a greater variety of signs because they lived longer and thus had a longer exposure to supersaturated water. Most of these fish had bubbles in the tail, and many had emphysema in the fins and the opercles. The cause of death is due to emboli (bubbles) in the blood stream blocking the capillaries and preventing normal blood flow through the gills and other tissues.

Much more extensive and severe signs of gas bubble disease developed at high O_2/N_2 ratios indicating that oxygen plays a significant role in forming external emphysema and lesions. Even those fish exposed to 130 percent total gas saturation (186.2 percent O_2/114.6 percent N_2) and dying within as little at 12 hours, had massive bubble formations on the head, opercles, tail, and fins when tested in supersaturated water with abnormally high oxygen levels. Exophthalmia, or popeye, where the eyes are driven out of the sockets from gas behind the eye, was also much more prevalent when the O_2/N_2 ratios were high.

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